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(54) Title: MEDICAL USE OF COMPOSITION COMPRISING FATTY ACIDS

(57) Abstract

A medicament for alleviating symptoms of multiple sclerosis is constituted by a combination of linolenic acid and linoleic acid (including their salts, esters, and amides) in specified percentage ratios. Such combination may include optionally an additional ingredient selected from C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids, in an amount which does not prevent the combination having potentially MS symptoms alleviating activity, as determined by testing such combination in an animal model. Pharmaceutical formulations and nutritional combinations are also described, as is a method for testing a chemical substance in an animal model for potential use in alleviating symptoms of multiple sclerosis.

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MEDICAL USE OF COMPOSITION COMPRISING FATTY ACIDS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a method for alleviating symptoms of multiple sclerosis by administration of a fatty acid combination; use of such combination in the manufacture of a medicament therefore, a composition of matter, pharmaceutical formulation and nutritional composition having potentially MS symptoms alleviating activity, and a method for testing a chemical substance for alleviating symptoms of multiple sclerosis in an animal model.

The present inventor has previously described a novel composition which comprises a combination of α-linolenic acid and linoleic acid, in defined proportions, which is potentially useful in enhancing memory, producing analgesia, regulating sleep, inhibiting senility symptoms, and in treating Alzheimer's disease and related dementia, and epilepsy (see U.S. Patents Nos. 4,851,431, 5,120,763, 5,288,755, 5,416,114 and 5,468,776). In U.S. Patent No. 5,194,448, there is described a pharmaceutical composition for use in the treatment of demyelinating diseases such as multiple sclerosis, consisting essentially of nervonic acid, as well as a process for treating such diseases by administering a composition including nervonic acid. The provision of such long chain fatty acids is based on a hypothesis that it could be beneficial to provide a pool of material vital for reassembly of the myelin sheath. The entire contents the foregoing U.S. patents are incorporated by reference herein.

Relationships between multiple sclerosis (MS) and lipids and fatty acids have been discussed in the past, with changes in lipid metabolism reported in the periphery for MS patients. In addition, changes in the level of cholesterol in the brain have also been described (Nicholas, H.J. et al., Central nervous system demyelinating diseases and increased release of cholesterol into the urinary system of rats, *Lipids*, **29** (1994) 611-617). Attempts to alleviate MS symptoms with compounds containing n-3 and/or n-6 fatty acids have shown only limited success (Field, E.J., *Multiple Sclerosis*, Springfield, Thomas CC, 1989). A study of essential fatty acid and lipid profiles in MS patients has been reported (Cunnane et al., Essential fatty acid and lipid profiles in plasma and erythrocytes in patients with multiple sclerosis, *Am. J. Clin. Nutr.*, **50** (1989) 801-806). MS is characterized by active degradation of central nervous system myelin, with clinical

symptoms dependent on the brain areas which undergo demyelination. While the etiology of MS is unknown, one of the major symptoms associated with MS is the deterioration of cognitive functions.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, in absence of a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids, an effective multiple sclerosis symptoms alleviating amount of a composition of matter which comprises (a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable, and (b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable, and wherein based on the combined weights of components (a) and (b), the composition of matter contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

In another aspect, the invention provides a method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, an effective multiple sclerosis symptoms alleviating amount of a pharmaceutical formulation which comprises at least one pharmaceutically acceptable substance selected from the group consisting of diluents, carriers and adjuvants except a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids; together with an active combination of: (a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable; and (b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of

linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable; and wherein, based on the combined weights of components (a) and (b), the combination contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

In still another aspect, the invention provides a method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, an effective multiple sclerosis symptoms alleviating amount of a nutritional composition including an orally ingestible carrier or diluent except a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids; together with an active combination of: (a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable; and (b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable; and wherein, based on the combined weights of components (a) and (b), the combination contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

In yet another aspect, the present invention provides use of the combination of linolenic and linoleic acids as defined herein, in the manufacture of a medicament for alleviation of symptoms of multiple sclerosis. In particular embodiments, such medicament may take the form of pharmaceutical formulations or nutritional compositions, as described herein.

In a further aspect, the invention provides a method for testing a chemical substance in an animal model for potential use in alleviating symptoms of multiple sclerosis, which comprises the steps of (1) treating laboratory rats with a sub-clinical dose of EAE such that (i) compared with EAE-treated rats, their life is prolonged to a period of more than 14 days and (ii) they nevertheless exhibit some EAE symptoms, including at least drunken gait with ataxia; and (2) treating rats resulting from step (1) with said substance, a positive test result being one in which rats treated in this step have

a statistically significant prolonged life compared with a control group from step (1) not treated with said substance.

For purposes of definition, the term "linolenic acid" without qualification, as used in the present specification and claims, means exclusively α-linolenic acid (9,12,15-octadecatrienoic acid). Also, reference in the present specification and claims to alleviating symptoms of multiple sclerosis is intended to relate also to alleviating symptoms of other demyelinating diseases where the context permits. Moreover, reference in the present specification and claims to "derivatives" of C_{8-18} saturated fatty acids and oleic acid is intended to include salts, esters and amides thereof.

DETAILED DESCRIPTION OF THE INVENTION

The combination of active components, or composition of matter, useful in the invention preferably consists of from about 14.3 to about 25.0% by weight of component (a) and about 85.7 to about 75.0% by weight of component (b), more preferably from about 16.3 to about 24.4% by weight of component (a) and about 83.7 to about 75.6% by weight of component (b).

In accordance with a particular embodiment of the invention, a special memory enhancement effect, and thus by inference a potential improved utility for the treatment of multiple sclerosis, has been noted when the composition of matter consists of from about 15.0 to about 24.5% (preferably about 20.0 to about 24.4%) by weight of component (a) and about 85.0 to about 75.5% (preferably about 80.0 to about 75.6%) by weight of component (b), or from about 16.7% (preferably about 18.2%) to about 22.2% by weight of component (a) and about 83.8% (preferably about 81.8%) to about 77.8% by weight of component (b); and particularly when the composition consists of either about 22.2% by weight of component (a) and about 77.8% by weight of component (b), or about 20.0% by weight of component (a) and about 80.0% by weight of component (b), or about 19.0% by weight of component (a) and about 81.0% by weight of component (b).

The preferred percentage proportions by weight are also of course applicable to the relationship between the at least one compound selected from linolenic acid and physiologically non-deleterious and hydrolyzable derivatives thereof, and the at least one

compound selected from linoleic acid and physiologically non-deleterious and hydrolyzable derivatives thereof (calculated as the free acids), in the nutritional compositions of the invention.

It is believed that the combination of linoleic and linolenic acids is the active principle *per se* which induces the effects mentioned, and thus it will be appreciated by skilled persons, that instead of the acids themselves, there may be derivatives of these acids which are both physiologically hydrolyzable (to the corresponding acids) and pharmacologically acceptable. Such derivatives may for example be selected from salts, esters and amides of the respective acids.

Among suitable salts there may be mentioned the ammonium, sodium, potassium, calcium and magnesium salts as salts with substituted mono- and di-substituted amines and the analogous saturated heterocyclic compounds containing an NH group in the ring, so long as the amines and the analogues in question are physiologically acceptable. As suitable esters there may be mentioned, for example, the ethyl and glyceryl esters. Amides of the acids may also be utilized, e.g. amides of the acids with substituted mono- and di-substituted amines and with the analogous saturated heterocyclic compounds containing an NH group in the ring, so long as the amines and the analogues in question are physiologically acceptable. It will be appreciated that the latter stipulation is necessary (in the case of the amine salts, the amides and their heterocyclic analogues) since it is to be expected that such derivatives will metabolize in the body to the desired acids and the starting amines or heterocyclic compounds. A person skilled in the art will of course know how to select a particular salt, ester or amide, so as to be physiologically hydrolyzable to the corresponding acid, and be pharmacologically acceptable.

The pharmaceutical formulations useful in the present invention may be adapted for oral, parenteral or rectal administration, and it may be in the form of dosage units. The diluents, carriers and adjuvants are those conventionally used in pharmaceutical and veterinary formulation.

For oral administration, the pharmaceutical formulations may be utilized as e.g. tablets, capsules, emulsions, solutions, syrups or suspensions. For parenteral administration, the formulations may be utilized as ampoules, or otherwise as suspensions, solutions or emulsions in aqueous or oily vehicles. The need for suspending, stabilizing and/or dispersing agents will of course take account of the fact of the

solubility or otherwise of the linoleic and linolenic acids, or of their derivatives used in the formulations, in the vehicles which are used in particular embodiments. Thus, for example, where the acids themselves are used, account will be taken of the fact that these have a relatively low water solubility and in general a relatively high oil solubility. The formulations may additionally contain e.g. physiologically compatible preservatives and antioxidants.

The pharmaceutical formulations may also be utilized as suppositories with conventional suppository bases such as cocoa butter or other glycerides. Alternatively, the formulations may be made available in a depot form which will release the active composition slowly in the body, over a preselected time period.

The nutritional compositions useful in the invention include as a necessary component an orally ingestible diluent or carrier; this may for example comprise a substance selected from sugar-based confectionery, a manufactured cereal, a fruit or vegetable product, a beverage or beverage concentrate, or any inert diluent, carrier or excipient known in the pharmaceutical art. It is intended generally that ingredients (a) and (b), as previously defined, may be used in nutritional compositions in any of the forms in which these are known and practiced in the art. Thus, the nutritional compositions may take the form of, e.g., sugar-based confectionery such as candies or chocolate, breakfast cereals, fruit or vegetable purees or beverages, other beverages (including those based on carbonated water), or beverage concentrates generally (including those in the form of e.g. powders, granules, flakes or crystals, which intended to be mixed with hot or cold water and/or milk).The nutritional compositions may also generally be in the form of powders, tablets, capsules, solutions. concentrates, syrups, suspensions, gels or dispersions. It will be evident that when the nutritional compositions take the form of dispersions or suspensions, it will usually be necessary to use an acceptable (i.e. non-toxic and otherwise suitable) dispersing or suspending agent, as is well known in the nutritional and pharmaceutical arts. When these compositions are utilized in the form of capsules, it will be evident that gelatin or other known suitable ingestible materials may be used for encapsulation.

The nutritional compositions useful in the invention may further include any of the known vitamins. Thus for example, such compositions (which may be, but need not be, in the form of aqueous suspensions) may comprise at least one water-soluble vitamin

selected from thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folic acid, cobalamin and ascorbic acid. Alternatively or additionally, such compositions may comprise at least one oil-soluble vitamin selected from retinol, calciferol, tocopherol and menadione. The nutritional compositions may also comprise in combined form at least one element selected from sodium, potassium, calcium, phosphorus, magnesium, chlorine and sulfur, and additionally or alternatively, at least one element selected from iron, copper, iodine, manganese, cobalt, zinc, molybdenum, fluorine, selenium and chromium. These compositions may also contain other natural or synthetic antioxidants.

The nutritional compositions useful in the present invention may also comprise other unsaturated fatty acids, such as for example those known to be metabolized in the body to prostaglandins, e.g. dihomo-gamma-linolenic acid, arachidonic and eicosapentaenoic acids, as well as physiologically compatible derivatives thereof, such as salts, esters and amides of such acids.

It is believed that a suitable dosage of the combination of linolenic and linoleic acid will be an amount within the range of 5-10 g per day, for an adult of average weight of 75 kg, but the physician will be able to determine the dosage rate according to the needs of an individual patient. This generalization does not preclude administering lower dosages, e.g. within the range of 0.1 to 5 g per day, or higher dosages above 5 g and up to 10, 20, 30, 40 or even 50 g per day, as may be determined by medical practitioners.

MEMORY TESTS USING THE LINOLENIC/LINOLEIC ACID COMBINATION

Although the present invention is primarily concerned with a method for alleviating symptoms of multiple sclerosis, nevertheless since memory and multiple sclerosis are both brain-related phenomena, and multiple sclerosis may be characterized *inter alia* by cognitive difficulties, it is believed that results of experiments on the effect on the memory of laboratory animals, of the linolenic/linoleic acid combination which is useful in the present invention, will serve to demonstrate the range of proportions and the preferred proportions in the linolenic/linoleic acid combination which are also of potential use in the treatment of multiple sclerosis. The results of comparative tests with structurally related acids are also recorded. For the experimental details of the memory tests on laboratory animals, see the above mentioned incorporated by reference US patents. The results are as stated in Table I, below.

TABLE I: MEMORY TESTS ON LABORATORY ANIMALS

	NUMBER OF TRIALS TO REACH CRITERION (10 secs.) WEEKS OF TREATMENT				
GROUP CODE	0	1	2	3	4
A (run 1)	19.6±3.7	19.0±3.3	20.3±2.7	19.1±2.9	18.5±2.5
A (run 2)	20.1±3.0	20.0±3.6	19.3±3.1	19.4±3.6	18.8±3.3
B	20.1±4.0	18.0±4.1	19.9±3.2	17.0±4.0	17.1±4.5
C	17.1±2.1	12.5±3.3*	10.7±4.1*	7.9±3.9*	5.6±2.5*
D (run 1)	18.5±2.0	9.3±2.6*	7.1±2.9*	6.1±2.8*	6.1±2.5*
D (run 2)	18.6±3.0	9.1±.3.0*	6.9±2.5*	6.0±2.8*	5.8±2.5*
E	19.1±3.7	14.2±2.3*	12.8±3.9*	9.6±3.0*	9.0±3.4*
F	19.5±2.6	16.1±3.5	11.2±1.1*	9.2±1.8*	7.9±1.0*
G	19.7±3.3	18.1±3.8	18.4±2.6	18.6±4.1	17.9±2.9
H	21.0±3.0	20.0±4.0	19.6±3.0	19.1±3.9	18.8±3.1
I	18.5±4.0	17.4±3.0	18.1±8.0	18.1±8.0	19.4±5.0
J	17.9±5.0	18.3±4.0	18.5±9.0	21.1±9.0	20.1±9.0
K	20.5±8.0	17.4±7.0	17.2±9.0	19.1±3.0	16.5±9.0
K'	20.1±7.0	16.9±2.0	16.3±10.0	18.2±5.0	17.6±8.0
L	19.7±9.0	20.1±5.0	20.3±9.0	22.8±9.0	21.9±9.0
L'	18.3±9.0	20.9±6.0	19.2±10.0	19.8±8.0	19.8±8.0
M	17.7±7.0	16.5±3.0	16.6±8.0	17.5±6.0	17.8±5.0
M'	18.5±7.0	19.6±8.0	20.6±8.0	20.4±11.0	20.1±8.0
N	18.1±5.0	17.5±4.0	19.7±11.0	19.4±10.0	18.7±11.0
O	17.1±6.0	18.5±7.0	20.8±6.0	19.8±11.0	20.6±12.0
Q	18.9±3.0	17.6±8.0	16.1±7.0	20.1±9.0	19.6±9.0
R	18.9±2.5	18.3±4.0	16.5±3.0	17.9±3.6	18.1±1.9
S	19.3±2.5	15.1±4.4	16.8±4.0	18.1±3.7	19.5±3.6
T	20.1±3.1	16.2±4.2	17.1±3.9	18.9±3.4	19.8±2.5
P:-	N.S.	0.01	0.01	0.01	0.01

*Statistically differs from Control (M ± SEM)

The nature of the administered substance in Table I is identified for each group in Table II, which states also the P value for each group.

TABLE II: KEY TO GROUP (SUBSTANCE) CODES AND GROUP P VALUES

CODE	SUBSTANCE	P
A	control	N.S.
B	25.0% linolenic acid	N.S.
C	22.2% linolenic acid	0.01
D	20.0% linolenic acid	0.001
E	18.2% linolenic acid	0.01
F	16.7% linolenic acid	0.01
G	15.4% linolenic acid	N.S.
H	14.3% linolenic acid	N.S
I	100.0% linolenic acid	N.S.
J	50.0% linolenic acid	N.S.
K	9.1% linolenic acid	N.S.
K'	6.3% linolenic acid	N.S.
L	4.8% linolenic acid	N.S.
L'	3.8% linolenic acid	N.S.
M	100.0% linoleic acid	N.S.
M'	83.3% linolenic acid	N.S.
N	90.9% linolenic acid	N.S.
O	95.2% linolenic acid	N.S.
Q	100.0% γ-linolenic acid	N.S.
R	20.0% γ-linolenic acid	N.S.
S	11.0% linolenic acid	0.01
T	11.0% γ-linolenic acid	N.S.

Note: in above compositions containing <100% linolenic or γ-linolenic acids, balance to make 100% based on the combined weights is linoleic acid

Discussion of results in Table I (memory or learning ability)

Groups B through H fall within the definition of the linolenic/linoleic acid combination useful in the present invention. The results show that in Groups C through F, in particular, injection of this composition has a statistically significant effect on the learning capacity of laboratory animals. In Groups B, G and H, the results, although not statistically significant, nevertheless show a tendency to improve learning capacity, compared with control Group A. Groups I through T are included for comparison purposes but do not fall within the definition of the combination of acids useful in the present invention. The contrast between the results in Groups R and D is particularly notable; it is self-evident that γ-linolenic acid cannot be substituted for linolenic acid with any expectation of obtaining a similar effect. Moreover, it is also evident from Table I that only a linolenic/linoleic acid combination within the defined range of proportions shows any tendency to improve the memory of laboratory animals; outside this range there is scarcely any difference from the control results.

THE EFFECT OF OTHER FATTY ACID AND NATURAL OIL CARRIERS ON LEARNING

When linolenic acid was added to corn oil, olive oil or sunflower oil, or when linoleic acid was added to linseed oil, in order that the linolenic acid/linoleic acid ratio (calculated as free acid) should be 1:4, such composition had no effect on learning. A similar absence of activity was noted in 1:4 linolenic acid/linoleic acid compositions, which included additionally as carrier free palmitic or stearic acid. Moreover, including oleic acid in the composition instead of palmitic or stearic acid gave inconclusive results. It is thus inferred that the presence of such saturated acids as palmitic and stearic acid (or more generally C_{8-18} saturated fatty acids), as well as oleic acid, or derivatives thereof (such as e.g. the above-mentioned oils which might otherwise have been possibly used as carriers), may adversely affect the desired biological activity of the active combination, and should not be used as carriers. However, the skilled person will be able to readily determine when such substances may be included in much smaller amounts as to not adversely affect the desired biological activity, and compositions (the potential activity of which could be verified e.g. by subjection to the animal model for MS as described

herein) containing such lesser quantities of these oily or fatty substances are deemed to be within the purview of the present invention.

Thus, included within the contemplation of the present invention, are compositions which comprise components (a) and (b) as defined herein and within the range of relative proportions defined herein, and which comprise also as a further component (c) at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids, in an amount which does not prevent the composition having potentially MS symptoms alleviating activity, as determined by testing such composition in an animal model; as well as a pharmaceutical formulation and nutritional composition comprising components (a), (b) and (c).

ANIMAL MODELS AND MS TESTING

As regards testing in laboratory animals for substances which will potentially alleviate symptoms of MS, although an ideal animal model of MS is unavailable, Experimental Allergic Encephalomyelitis (EAE) is considered the preferred model (Werkele, H., Lymphocyte Traffic to the Brain, in W.M. Pardridge (Ed.), *The Brain Blood Barrier*, Raven Press, New York, 1993, pp. 67-83).

However, the main methodological problem of investigating EAE animals is that the onset of the illness is very fast and deterioration is swift. EAE animals, once ill, do not survive more than 2 weeks. I have successfully replicated the method for induced EAE as demonstrated earlier by Nicholas et al., *loc cit.* In addition, to enable measurement of behavioral variables, and as described below, I have administered a diluted dose of the agent to incude EAE (dEAE), which created partially-sick animals. This technique enabled creation of sick rats with a reduction in the severity of symptoms. Behaviorally, rats which received a full EAE dose exhibited fast deteriorating motor activity. They showed progressive ataxic motion, weaving, drunken gait and ineffective movement. In addition, they had static tremor and shivering. They did not react to a loud noise. In the last stage, they were unable to move and shivered. In comparison, the rats which received dEAE dose also showed motor problems but much less severe. They exhibited drunken gait with ataxia, but the motion was effective. The degree of shivering was much less harsh and they reacted to a loud noise. Rats who received a full dose in a

preliminary study survived from 11-l4 days compared to rats which received the diluted dose who survived 26-27 days (n=10 rats per group).

Described herein are the protocol and results of a study which investigated the effects of a 1:4 α-linolenic acid/linoleic acid mixture (designated SR-3) on (a) a spatial learning task (measured by the Morris Water Maze) and a passive avoidance task, (b) fatty acids profile, and (c) cholesterol level. The latter two were measured by gas chromatography. Also, the status of the myelin in the frontal cortex of treated and untreated rats was measured by Luxol Fast Blue Technique (Wakefield, A.J., et al., Immunohistochemical study of vascular injury in acute multiple sclerosis, *J. Clin. Pathol.*, 47 (1994) 129-133) and served as an index of the effectiveness of the EAE model (full dose and diluted dose) and the effectiveness of the SR-3 treatment. In this study we used the semiquantitative method of Yu, G.S.M., et al., Effect of prenatal iron deficiency on myelination in rat pups, *Am. J. Pathol.*, **125** (1986) 620-624, where the amount of myelin was evaluated on a 5 point scale.

The use of SR-3 in the present tests in merely exemplary and is of course without prejudice to the broader scope of the 1:4 α-linolenic acid/linoleic acid combination useful in the present invention and as defined herein.

Materials and Methods

Test material:

α-linolenic (0.92 g/ml) and linoleic (0.90 g/ml) free fatty acids, both 99% pure (as evaluated by capillary gas chromatography), were purchased from Sigma (L2367 and L1376). The test substances were stored in the dark at 4° C. A fresh stock solution (1 ml) was prepared every 3 days by mixing 0.40 ml of 1:4 α-linolenic acid/linoleic acid mixture, mineral oil (0.59 ml) and α-tocopherol (0.02 ml).

Animals:

Male Lewis rats (230-270g body wt.) were used. They were housed individually in hanging, stainless steel, wire-mesh cages in a well-ventilated room that was air-conditioned by means of a system designed to maintain the room temperature at an average of 22° C and a relative humidity of about 45%. The room was illuminated by fluorescent light that simulated the spectrum of the sun (Vita-Lite; Dura-Test; Clifton

NJ) to permit an artificial 24h cycle of 12h of light daily (from 6 am to 6 pm). Tap water and Altromine C-1000 diet were available ad libitum The diet contained 5.1% fat.

Summary of experimental design:

Each rat in the first experimental group (n=20) was injected intradermally at 6 sites in both hind feet pads with a 0.25 ml suspension of 0.5 mg (wet weight) of whole guinea pig spinal cord plus 3.0 mg of *M. Tuberculosis* per ml of Incomplete Freund's Adjuvant. Immediately after the EAE treatment, 10 rats received SR-3, and 10 rats received mineral oil (+ α-tocopherol). Subsequently they became very ill, exhibited flaccid paralysis of the hind limbs. The brains of these rats were analyzed by Luxol method. No behavioral studies were performed on these rats.

The second group of 60 rats received the EAE inducing mixture, but the total dose was decreased to 0.12 ml (dEAE dose). Preliminary studies showed that at this dose level rats survived 26 - 27 days. Twenty rats from this group received a daily injection of SR-3 for 3 weeks, 40 mg/kg ip. Another 20 rats received an injection of mineral oil (+ α-tocopherol) and served as a control group, and the remaining 20 rats received saline. The third group of 60 rats received a saline injection in the location of hind pads, and were divided into SR-3 treatment or mineral oil (+ α-tocopherol) treatment, or saline (n=20 per group). In order to establish a base-line for Luxol method, 10 rats received SR-3 and 10 rats received mineral oil treatment for 3 weeks, and their brains were analyzed for Luxol.

On day 17 rats which received dEAE dose, were tested in an activity meter and their body temperature was measured. On day 18, after the beginning of treatment (SR-3 or mineral oil), all rats were tested in the Morris Water Maze for 3 days. On day 21 all were tested on the Passive Avoidance Apparatus. On day 22 the rats were sacrificed. The brain of half of each group was analyzed for fatty acids and cholesterol, and the other half for Luxol fast blue study.

Morris Water Maze:

The Morris water tank, a circular tank (110 cm in diameter), was filled with water (to the level of 40 cm), which was made opaque by the addition of powdered milk, so that rats swimming in the tank were unable to see an escape platform (7.5 cm in diameter) submerged 2 cm below water level. Each animal was released facing the wall

in one of four predetermined starting points each separated by 90 cm around the inner perimeter. While the rat was in the tank, it was able to observe the contents of the room. Special care was given to keep things in the room in the same location. The rat could navigate in the tank only by external cues. Each rat was tested 8 times per day in the tank. The order of the starting point was determined by random selection, to prevent possible effects of a magnetic field. Each rat was allowed 120 sec. to find the platform, with an interval of 20 sec. between trials. The maximum duration of the test for each rat was 16 min., and three rats were tested each hour. The rats were tested on 3 consecutive days. During this period, the platform was in the same location in the tank. For each of the 24 trials (eight trials on each of three days), the latency to reach the platform was recorded. A cutoff criterion, defined as the first successful trial with a maximum latency of 10 sec. without any increase in latency on a later trial, was used to calculate an index of learning ability (rate of learning) for each group. In addition, the length of the swimming path of each rat was recorded. (Yehuda, S. et al., Modulation of learning, pain thresholds, and thermoregulation in the rat by preparations of free purified linolenic and linoleic acids: Determination of optimum n-3 to n-6 ratio, *Proc. Natl. Acad Sci. USA*, **90** (1993) 10345-10349.).

Luxol staining for myelin:

Rats were anesthetized with sodium pentobarbital and their brains were removed *en bloc*. Each brain was fixed in 2% glutaraldehyde for 4 hours, and then transferred to buffer for further processing. Paraffin-embedded tissue was cut at 6μ and stained with Luxol fast blue. The dye Luxol fast blue was of the sulfonated copper phthalocyanine type. The degree of myelination was graded semiquantitatively by microscope examination of the sections, independently by two pathologists, each having no knowledge either of results obtained by the other, or the type of treatment the rat received. The degree of agreement between the two pathologists was 97%. A grading system of 0 - 5 was designated as follows: 0, total absence of blue staining; 1, faint blue staining; 2, mild patchy confluent blue staining with focally uncomplicated areas; and 5, complete, confluent, blue staining (Yu, et al. *loc cit.*, 1986).

Synaptosomes and determination fatty acids and cholesterol:

Synaptosomes were prepared as suggested by Whittler, V.P. et al., "The subcellular fractionation of brain tissue with special reference to the preparation of

synaptosomes and their component organelles" in R. Fried (Ed.), *Methods in Neurochemistry*, NY, Marcel Dekker, 1972, pp. 1-52. Brain tissues were homogenized on ice in 0.32M sucrose, pH 7.0, and centrifuged at 23,000 x g for 20 min. at l° C. The supernatant was discarded, the pellet resuspended in 6 ml of 0.32M sucrose, applied to a discontinuous sucrose gradient (0.32M, 0.8M. and l.2M) and centrifuged at 100,000 x g (Model L8-55, Beckman) for 60 min. at 1°C. Synaptosomes were removed from the 0.8-l.2M sucrose interface with Pasteur pipettes, diluted 1:1 with distilled water and centrifuged at 23,000 x g for 20 min. at 1°C. The resulting pellet was resuspended in 1.0 ml of 0.32M sucrose, rehomogenized and stored at -70°C until analyzed. Lipids were extracted from the membranes in a vial containing 15 ml chloroform/methanol (1:2 vol./vol.) according to Polch-Pi, I., et al., A simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem.*, **226** (1957) 497-509. Recovery of synaptosomes was greater than 87% and the purity was determined by electron microscopy. Lipids were analyzed for fatty acids composition by gas chromatography (Varian, SP-2330 Supelco column, BPx70 Capillary column 50m, 0.33mm ID, Model DB-23 SGE). The results from the gas chromatography (GC) were verified by mass spectrometry (4030 Finnigen-GS-MS, Sunnyvale, California). Cholesterol was analyzed by the same GC, using a STIB-5 Supelco column, fillary column, 15 mm, 0.32 ID. Fatty acid quantification was done by comparison with GLC standard mixtures, GLC3O-4-7040, AOCS-4-l019, and GLS60-4-7043 (Supelco, Bellefonte, PA). The following variables were calculated: total fatty acids, FA ratio (the ratio between saturated and unsaturated fatty acids) and the cholesterol level.

Measurement of motor activity:

The level of motor activity was assessed in an open field apparatus (75 cm x 75 cm), by recording the number of horizontal (infrared photobeam crossing) and rearing (determined from videotaping) movements made during the 15 min. sessions (Brandeis, R. et al., The use of the Morris Water Maze in the study of memory and learning, *Int. J. Neurosci.*, **48** (1984) 29-69.)

Passive avoidance:

The Passive Avoidance box consisted of a bright and dark compartment. During the training trial, (day 20) the rats were placed into the bright compartment. After the

rats entered the dark compartment a shock was delivered (0.5 mA. 3 s). Twenty four hours later the rats were again placed in the bright compartment and the latency to re-enter the dark compartment was measured (maximum duration: 360 s). Short entry latencies indicated poor avoidance learning.

Body weight:

The rats were weighed on day 0 and day 17.

Body temperature:

Body temperature was measured by a telethermometer (YSI Telethermometer, Model 43TA, Yellow Springs, Ohio).

Statistical Analysis:

There was no difference between the mineral oil group and the saline group. Therefore, all statistical analyses were performed between the SR-3 group and the mineral oil group. All results are expressed as means with standard deviations (SD). The statistical significance of the mean differences was determined by ANOVA and Student's t-Test.

Results

The most surprising result was the extension of the survival span. The 10 rats that received the full dose of the EAE induction survived 11.4±1.3 days. When SR-3 was administered to EAE rats, they survived 17.1±1.1 days although the severity of the symptoms was unchanged. ($t_{(18)}$=10.718, $p< 0.01$). When the diluted dose of the EAE inducing agent was administered to 10 rats who did not receive any treatment except for food and water, they survived 24.5±1.3 days. Animals treated with SR-3 survived from 29.3±1.2 days ($t_{(18)}$=5.286, $p< 0.01$).

The dEAE rats were lighter. The average body weight gain during the first 17 days was 2.0±0.4 g in the group which received mineral oil (+ α-tocopherol) and 2.7± 0.6 g ($t_{(18)}$=3.1, $p< 0.01$) in the group which received SR-3. For rats receiving saline injection, the average body weight was 3.1±0.9 and 3.0±0.8 for SR-3 treated rats.

Another surprising result was the ability of SR-3 to restore some of the learning deficits which followed the dEAE treatment. The effectiveness of the dEAE treatment was evaluated and confirmed by Luxol fast blue staining. Control rats showed

myelination of the frontal cortex at 4.9 (± 0.31 point). Control rats which received SR-3 showed a score of 4.9 ±0.01. Full EAE dose with mineral oil induced a score of 0.4±0.1, and a full dose with SR-3 induced a score of 0.7±0.15. dEAE rats which received mineral oil were at 1.9 (±0.73) point and the SR-3 treatment in that group increased the myelination to 3.4± 0.51. [Two-way ANOVA, main effect dEAE treatment ($F(1,36) = 344.2$, $p < 0.001$), main effect SR-3 treatment ($F(1,36) = 74.2$, $p < 0.001$), Interaction ($F(1,36) = 63.22$, $p < 0.001$)]. Scheffe's analysis showed that the "dEAE treatment" group which received SR-3 was significantly different from all the other experimental groups ($\alpha = 0.05$). Moreover, a highly significant t-test between the means of dEAE groups with and without SR-3 was found ($t_{(18)}=8.78$, $p < 0.001$). SR-3 was able to restore some of the damage caused by the dEAE treatment, but at the dose level in this experiment, SR-3 was unable to effect full recovery to normal level.

Only dEAE treated rats and their control groups were tested in the behavioral tests. Full dose EAE treated rats were not tested. For every behavioral variable, a 2-way ANOVA was calculated. All interactions were found significant.

Similar results to the biochemical findings were obtained in the learning data. The dEAE treatment induced severe learning deficits. While SR-3 was able to reduce the number of trials for normal rats to reach criterion by one-third, i.e. from 18 to 6 ($t_{(18)}=9.5$, $p < 0.001$), among dEAE treated rats the reduction in number of trials was about 22 to 15 ($t_{(18)}=4.32$, $p < 0.001$) (Table III). The "Swim Span" was also reduced by the SR-3 treatment: for the control rats from 500 cm to 370 cm ($t_{(18)}=7.151$, $p < 0.001$) and for dEAE rats from l232 cm to 810 cm ($t_{(18)}=16.6$, $p < 0.001$). However, the dEAE rats (which did not receive SR-3) covered a much longer distance (2.5 times longer) (Table III). While the SR-3 treatment did not improve the Passive Avoidance learning for the control rats, the treatment had significant effects for the dEAE rats, which improved from 100 to 280 sec ($t_{(18)}=17.8$, $p < 0.001$), but did not reach normal levels. (Table III). No correlation was found between body weight and learning capacity. The SR-3 treatment did not affect motor activity for the control rats, but did rehabilitate the level of the impaired motor activity of the dEAEL rats, in that the line crossing was increased from 427 to 610 ($t_{(18)}=9.61$, $p < 0.01$) and the rearing from 20 to 24 ($t_{(18)}=2.16$, $p <$

0.005) (Table III). It should be remembered that (a) despite their motor problems the dEAE rats swam a greater distance, and that (b) passive avoidance learning does not require motor activity. As regards the body temperature, EAE rats exhibited hypothermia of 33.1° C, compared to 36.8°C for the control rats ($t_{(18)}$=14.3, $p< 0.001$); SR-3 increased the body temperature to 35.9°C ($t_{(18)}$=9.39, $p< 0.001$), only slightly below the normal temperature level (Table III).

Table III. Behavioral Effects of dEAE treatment.

	CONTROL		dEAE	
	Mineral Oil	SR-3	Mineral Oil	SR-3
No. of trials to reach criteria	18.5±3.1	6.1±2.5	22.1±3.0	15.8±3.5
Swimming span (cm)	500±35.4	370±45.3	1232±50.4	810±631
Passive Avoidance (max. 360 sec)	325±3.5	353±6	100±20	280±25.6
Line crossing	750±30	733±26.3	427±40	610±45
Rearing	65±5	68±7	20±3	24±5
Body temperature	36.8±0.7	37.1±0.8	33.1±0.5	35.9±0.8

The profile of fatty acids of the dEAE treated rats was significantly different from the profile of the control rats ($p <0.01$). There was an increase in the level of the 16:0 fatty acid, and there was a decrease in the level of 18:2(n-6), 18:3(n-3), 20:3(n-6), and 20:4(n-6) ($p <0.01$). The treatment with SR-3 corrected those changes ($p <0.05$). The fatty acids profile of dEAE rats differs from that of normal rats, mostly noted in a reduction in total fatty acids in the synaptosome (2.5 to 1.8, $p <0.01$), an increase in the ratio of saturated/unsaturated fatty acids (1.1 to 1.3, $p <0.05$), and a significant increase in the cholesterol level (6.8 to 8.6, $p<0.01$). Treatment by SR-3 was able to modify the abnormal profile (Table IV).

Table IV. Fatty acid composition of frontal cortex synaptosomes in EAE rats with or without SR-3 treatment.

	CONTROL		dEAE	
	Mineral Oil	SR-3	Mineral Oil	SR-3
14:0	1.5±0.6	1.3±0.5	1.8±0.9	1.2±0.6
16:0	21.5±2.6	21.9±2.68	23.9±2.8	21.6±2.8
18:0	26.4±0.9	22.1±1.4	27.1±1.3	25.3±1.8
18:1 (n-9)	24.5±1.9	25.9±2.2	26.1±1.8	24.8±2.0
18:2 (n-6)	0.9±0.4	1.9±0.6	0.5±0.4	1.2±0.5
18:3 (n-3)	1.2±0.5	3.7±1.8	0.6±0.5	1.0±0.4
20:0	0.2±0.1	0.04±0.01	0.2±0.1	0.1±0.1
20:3 (n-6)	3.0 ±0.8	2.5±0.8	2.0±0.5	2.2±0.6
20:4 (n-6)	4.2±1.3	3.1±1.3	3.3±1.4	3.5±1.8
21:0	0.3±0.1	0.3±0.1	0.7±0.2	0.3±0.1
22:1	2.5±0.7	2.0±1.0	2.9±1.2	2.4±1.5
22:4 (n-6)	2.6±0.4	2.0±1.5	1.6±0.7	1.8±1.0
22:6 (n-3)	11.1±1.0	14.0±1.7	9.2±0.8	14.5±1.2
Total FA	2.5±1.0	3.5±1.4	1.8±0.9	2.2±0.8
Ratio S/US	1.10	0.87	1.30	1.03
Cholesterol	6.8±2.1	4.4±1.6	8.6±2.0	6.9±1.4

Note. Individual FA values are expressed as percentages of total FA composition and given as mean ±SD (n=l0). Total FA is expressed as percent of frontal cortex weight. Cholesterol is expressed as promil of frontal cortex weight.

Discussion

The changes in the cholesterol level are of particular interest. The importance for studying the cholesterol level follows Holman, et al., Deficiencies of polyunsaturated fatty acids and replacement by nonessential fatty acids in plasma lipids in multiple sclerosis, *Proc. Natl. Acad. Sci. USA*, **86** (1989) 4720-4724, who claimed that there is a decrease in the "membrane fluidity index" among MS sufferers. Since cholesterol in the membrane decreases the fluidity index of the membrane, our finding of a significant increase in the cholesterol level would support that view. However, the role of

cholesterol in MS has not been extensively studied, although all studies have indicated an increase in brain cholesterol. (Nicholas, et al., *loc cit.*; Syndyk, R.. et al. The relationship between melatonin secretion and serum cholesterol in patients with multiple sclerosis, *Int. I. Neurosci.*, **76** (1994) 81-86.) The increase in the brain cholesterol level might result from two different mechanisms: either (1) an overproduction of cholesterol in the brain, or (2) low density lipoprotein (LDL), which is the major carrier of plasma cholesterol, may enter the brain in MS due to modification of the blood-brain barrier (Newcomb, J., et al., Low density lipoprotein uptake by macrophages in multiple sclerosis plaques: Implications for pathogenesis, *Neuropathol. Appl. Neurobiol.*, **20** (1994) 152-162.).

Lipids and fatty acids are major components of myelin. Therefore, changes in the fatty acid metabolism or in the bioavailability of fatty acids and lipids in the brain will induce the modification of myelin and of the neuronal membrane structure. It is interesting that in 1983, it was reported that deficiency in n-3 fatty acids is associated with an increased susceptibility to EAE, requiring a smaller dose of antigen to induce it (Dhopeshwarkar, G.A., Nutrition and Brain Development, NY, *Plenum*, 1983).

The possible link between the profile of fatty acids and the cholesterol level to learning and cognitive capacity is the optimal level of neuronal membrane function, expressed as the "membrane fluidity" index. An optimal index allows the exchange of ions between the inside and outside of the membrane. This process is crucial for the transfer of neuronal information and to proper activity of membrane receptors. Cholesterol induces rigidity of the membrane, and essential fatty acids increase the fluidity index.

The Morris water maze and passive avoidance are two behavioral techniques which enable selected assessments of learning to be made. They are widely used, both in evaluating learning deficits induced by brain lesions and pharmacological agents, as well as for evaluating new 'learning enhancer' drugs (see e.g. Cunnane et al., *loc cit.* 1989). It is clear, however, that these methods examine different aspects of learning abilities. The Morris water maze tests the spatial capacity, whereas the passive avoidance test reflects response inhibition in the presence of noxious stimuli. It is interesting to note that both learning types suffer in animals of the diluted EAE condition. These results may indicate that the effects of the EAE condition are not restricted to one type of learning alone.

The results of this study indicated that SR-3 was able to restore the EAE damage to the myelin, to the changes in fatty acids profile, to the cholesterol level, and to the learning performances. Changes in myelin content and in fatty adds profile of brain synaptosomes may interfere with the activity of ionic channels and with the transfer of neuronal information, while changes in cholesterol may cause membrane hardening. Both mechanisms might explain decreases in learning capacity.

Most investigations of EFA effects on EAE animals have studied the effects of γ-linoleic acid (which is derived from Evening Primrose oil or from a fungal source) on peripheral markers such as lymphocytes or red blood cells. To my knowledge, the present disclosure is the first relating to the effect of (α-linolenic + linoleic) acids on such central variables as myelin and the synaptosomal FA profile. The results of this study together with those from previous studies demonstrate that SR-3 has profound effects on brain biochemistry and on cognitive functions. This study also demonstrated that a diluted induction dose of EAE is appropriate for the study of cognitive effects in that model. The changes in the level of myelin (as measured by Luxol), the changes in the fatty acids profile, and the motor symptoms observed after the dilute EAE treatment all contribute to the validity of the dilution procedure as providing a faithful model of the early stages of MS, confirmed by the changes in the level of myelin which are comparable to changes observed in MS, and by changes in the fatty acids profile.

While the present invention has been particularly described with reference to certain embodiments, it will be apparent to those skilled in the art that many modifications and variations may be made. The invention is accordingly not to be construed as limited in any way by such embodiments, rather its concept is to be understood according to the spirit and scope of the claims which follow.

CLAIMS

1. A method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, in absence of a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids, an effective multiple sclerosis symptoms alleviating amount of a composition of matter which comprises (a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable, and (b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable, and wherein based on the combined weights of components (a) and (b), said composition of matter contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

2. A method according to claim 1, wherein said composition of matter contains, based on the combined weights of components (a) and (b), about 16.3% to about 24.4% by weight component (a) and about 83.7% to about 75.6% by weight component (b).

3. A method according to claim 2, wherein said composition of matter contains, based on the combined weights of components (a) and (b), substantially 20.0% by weight component (a), balance to make 100% component (b).

4. A method according to claim 2, wherein said composition of matter contains, based on the combined weights of components (a) and (b), substantially 22.2% by weight component (a), balance to make 100% component (b).

5. A method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, an effective multiple sclerosis symptoms alleviating amount of a pharmaceutical formulation which comprises at least one

pharmaceutically acceptable substance selected from the group consisting of diluents, carriers and adjuvants except a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids; together with an active combination of:

(a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable; and

(b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable;

wherein based on the combined weights of components (a) and (b), said combination contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

6. A method according to claim 5 wherein said combination contains, based on the combined weights of components (a) and (b), about 16.3% to about 24.4% by weight component (a) and about 83.7% to about 75.6% by weight component (b).

7. A method according to claim 6, wherein said combination contains, based on the combined weights of components (a) and (b), substantially 20.0% by weight component (a), balance to make 100% component (b).

8. A method according to claim 6, wherein said combination contains, based on the combined weights of components (a) and (b), substantially 22.2% by weight component (a), balance to make 100% component (b).

9. A method for alleviating symptoms of multiple sclerosis in a mammal which comprises administering to the mammal, an effective multiple sclerosis symptoms alleviating amount of a nutritional composition including an orally ingestible carrier or

diluent except a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids; together with an active combination of:

(a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable; and

(b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable;

wherein based on the combined weights of components (a) and (b), said combination contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

10. A method according to claim 9 wherein said combination contains, based on the combined weights of components (a) and (b), about 16.3% to about 24.4% by weight component (a) and about 83.7% to about 75.6% by weight component (b).

11. A method according to claim 10, wherein said combination contains, based on the combined weights of components (a) and (b), substantially 20.0% by weight component (a), balance to make 100% component (b).

12. A method according to claim 10, wherein said combination contains, based on the combined weights of components (a) and (b), substantially 22.2% by weight component (a), balance to make 100% component (b).

13. A method according to claim 10, wherein said combination contains, based on the combined weights of components (a) and (b), substantially 19.0% by weight component (a), balance to make 100% component (b).

14. Use of a combination of the following components (a) and (b) in the manufacture of a medicament for alleviation of symptoms of multiple sclerosis:

(a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable; and

(b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, the salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable;

wherein based on the combined weights of components (a) and (b), the combination contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b).

15. Use according to claim 14, wherein said medicament is further characterized by the absence of a carrier or diluent which comprises at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids.

16. Use according to claim 15, wherein said medicament is in the form of a pharmaceutical formulation which comprises at least one pharmaceutically acceptable substance selected from the group consisting of diluents, carriers and adjuvants.

17. Use according to claim 15, wherein said medicament is in the form of a nutritional composition including an orally ingestible carrier or diluent.

18. Use according to ant of claims 14-17, wherein based on the combined weights of components (a) and (b), the combination contains about 14.3 to about 25.0% by weight of component (a) and about 85.7 to about 75.0% by weight of component (b), preferably 15.0 to about 24.5% by weight of component (a) and about 85.0 to about 75.5% by weight of component (b), more preferably from about 16.3 to about 24.4% by weight of component (a) and about 83.7 to about 75.6% by weight of component (b),

most preferably about 16.7% to about 22.2% by weight of component (a) and about 83.8% to about 77.8% by weight of component (b).

19. Use according to claim 18, wherein based on the combined weights of components (a) and (b), the combination contains about 18.2% to about 22.2% by weight of component (a) and about 81.8% to about 77.8% by weight of component (b).

20 Use according to claim 19, wherein based on the combined weights of components (a) and (b), the combination contains about 22.2% by weight of component (a) and about 77.8% by weight of component (b).

21. Use according to claim 19, wherein based on the combined weights of components (a) and (b), the combination contains about 20.0% by weight of component (a) and about 80.0% by weight of component (b).

22. Use according to claim 19, wherein based on the combined weights of components (a) and (b), the combination contains about 19.0% by weight of component (a) and about 81.0% by weight of component (b).

23. A composition of matter which comprises (a) at least one compound selected from the group consisting of linolenic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linolenic acid being both physiologically hydrolyzable and pharmacologically acceptable, and (b) at least one compound selected from the group consisting of linoleic acid and salts, esters, and amides thereof, calculated as the free acid, said salts, esters, and amides of linoleic acid being both physiologically hydrolyzable and pharmacologically acceptable, and wherein based on the combined weights of components (a) and (b), said composition of matter contains about 13.0% to about 27.5% component (a) and about 87.0% to about 72.5% component (b), and which composition of matter comprises also (c) at least one member of the group consisting of C_{8-18} saturated fatty acids, oleic acid and derivatives of these acids, in an amount which does not prevent the composition having potentially MS

symptoms alleviating activity, as determined by testing such composition in an animal model.

24. A pharmaceutical formulation which comprises at least one pharmaceutically acceptable substance selected from the group consisting of diluents, carriers and adjuvants, and components (a), (b) and (c), as defined in claim 23.

25. A nutritional composition including an orally ingestible carrier or diluent, and components (a), (b) and (c), as defined in claim 23.

26. A method for testing a chemical substance in an animal model for potential use in alleviating symptoms of multiple sclerosis, which comprises the steps of:
(1) treating laboratory rats with a sub-clinical dose of EAE such that (i) compared with EAE-treated rats, their life is prolonged to a period of more than 14 days and (ii) they nevertheless exhibit some EAE symptoms, including at least drunken gait with ataxia;
(2) treating rats resulting from step (1) with said substance, a positive test result being one in which rats treated in this step have a statistically significant prolonged life compared with a control group from step (1) not treated with said substance.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00366

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 37/00; A61K 31/20
US CL : 514/558

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/558

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3,993,775 A (WILLIAMS) 23 November 1976, see claims 1-10.	1-26
X	US 4,386,072 A (HORROBIN et al.) 31 May 1983, see claim 1 and column 2, lines 49-54.	1-26
X	US 5,194,448 A (COUPLAND et al.) 16 March 1993, see claims 1-14.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 FEBRUARY 1998	Date of mailing of the international search report 27 FEB 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DWAYNE C. JONES Telephone No. (703) 308-1235

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00366

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

IFICDB, USPATFULL, WPIDS, MEDLINE, EMBASE, TOXLIT, BIOSIS search terms: fatty(3a)acid#, multiple sclerosis, linolenic or linoleic or myristic or oleic or steric, administer######, c9 and higher acids, c9-c30 fatty acids, decanoic acid, fatty acids